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Hydrolysis kinetics in anaerobic digestion of waste activated sludge enhanced by α-amylase

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ABSTRACT

The thermodynamics of waste activated sludge (WAS) hydrolysis process enhanced by additional α-amylase were evaluated in this paper. The effects of enzyme dosage and temperature on WAS hydrolysis were further discussed based on the analysis of variance (ANOVA). The results showed that the rate constant of α-amylase hydrolysis process (α-amylase = 0.06 g/g dry sludge (DS)) increased from 0.106 to 0.215 h^{-1} with the temperature increasing from 40 to 70 °C, and the reaction activation energy for VSS hydrolysis reduced from 62.72 kJ/mol (control test) to 20.19 kJ/mol (α-amylase treatment). Kinetics analysis indicated that the enzymatic hydrolysis process well fitted the first-order kinetics model at 50 °C, and the conversion coefficients (α) of VSS to soluble chemical oxygen demand (SCOD), carbohydrates and NH_4^+–N was found to be 0.266, 0.043 and 0.038, respectively. The model could certainly provide a dynamical description of mechanism and would be benefit to analysis, design, optimization and control of enzymatic hydrolysis process of WAS.

1. Introduction

Anaerobic digestion, in which three steps (hydrolysis, acidification and methanogenesis) are generally involved, is widely applied to stabilize organic matters of the sludge and thereby prevent or slow the release of harmful chemicals into environment [1]. As the first step, the hydrolysis of sludge has received more attention, since the hydrolysis of particulate organic matter to soluble substance is believed to be the rate-limiting step of anaerobic digestion [2].

Enzymes could be applied to promote the disintegration of large sludge particles during sludge hydrolysis process, producing more surface area for microbes to attach, which led to a high efficient degradation of the sludge [3]. It was reported that lysozyme could be used for the destruction of the cell walls of Gram positive cells [4,5]. While for Gram negative cells, a combination of lysozyme and EDTA was found to be effective for the release of lipopolysaccharide molecules in membranes into the solution [6].

The hydrolysis of sludge enhanced by enzyme has been investigated for the last three decades and a number of enzymes, such as protease, amylase and endo-glycanases were reported to play an important role in the hydrolysis of biodegradable particulate organic matter [7,8]. Compared with other chemical conversion process, the enzymatic hydrolysis of sludge has advantages in higher yields, minimal byproduct formation, low energy requirements, and mild operation conditions [9]. Roman et al. [7] investigated the application of hydrolytic enzymes (cellulase and pronase E) to decrease solids, and resulted in 80% reduction in solids and 93% removal of particulate COD. The efficiency of industrial-scale anaerobic digestion was also improved with the addition of glycosidic enzymes [10].

Kinetic models for hydrolysis can be used to describe the relationship among the principal state variables and explain the behavior of hydrolysis quantitatively. In addition, it can provide useful information for analysis, design and operation of a hydrolysis process. Numerous studies emphasized on enzymatic hydrolytic process, and some kinetic models have been used to describe the hydrolytic behavior. South et al. [11] developed a kinetic model based on enzyme-adsorption, which indicated that the hydrolysis rate of lignocellulose increased with the increase of hydrolytic enzymes concentration and the availability of adsorption sites. He et al. [12] studied the kinetics of

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enzymatic hydrolysis of polysaccharide-rich particulates, and applied a simplified Chen–Hashimoto model to fit the hydrolysis data. The kinetic models have also been applied to describe sludge hydrolysis process. Li et al. [13] applied multivariable linear regression method to establish kinetics model for combined (alkaline + ultrasonic) sludge disintegration, and inferred that pH had the most significant effect on sludge disintegration. Mu et al. [14] systemically investigated anaerobic hydrogen production by mixed anaerobic cultures, and adopted Michaelis–Menten equation, Logistic model and modified Gompertz equation to describe the kinetics of sludge hydrolyis process.

However, there are very limited studies about the kinetic models for enzymatic hydrolysis of WAS. As reported in our previous study [8], α-amylase could strongly enhance the WAS hydrolysis. The construction of kinetic models for hydrolysis process enhanced by α-amylase would be very important for its further application. Therefore, this paper was mainly focused on the development of thermodynamics model and the analysis of dynamic behaviors of substances conversion during WAS hydrolysis process enhanced by α-amylase.

2. Materials and methods

2.1. Sludge and enzyme

WAS were collected from the secondary sedimentation tank of the second municipal wastewater treatment plant in Changsha, China. Fresh sludge was concentrated by settling for 4 h, further filtered through a 0.71 mm metal sieve and then stored at 4 °C until the experiments. The characteristics of the WAS were as followings: pH 6.74 ± 0.15, total chemical oxygen demand (TCOD) 7426 ± 254 mg/L, SCOD 140 ± 10 mg/L, total solid sludge (TSS) 7824 ± 200 mg/L, volatile suspended solids (VSS) 5360 ± 80 mg/L.

The α-amylase was purchased from Jiehui biotechnology Ltd. in Shanghai, China. The activity of α-amylase was 6000 U g^{-1}, and the optimal temperature was 50–70 °C.

2.2. Enzymatic hydrolysis tests

Experiments of the effect of α-amylase on WAS hydrolysis were conducted in a set of 250 mL flasks containing 100 mL WAS and α-amylase with various dosages (0, 0.03, 0.06, 0.12 and 0.18 g/g DS). Oxygen was removed from the headspace by nitrogen gas sparging for 4 min to maintain strict anaerobic condition. The flasks capped with rubber stoppers were agitated in a water-bath shaker at 100 rpm and 50 °C for 4 h.

Four batches of experiments were undertaken to investigate the effect of temperature on enzymatic hydrolysis of WAS. The enzymatic hydrolysis tests were carried out in four identical 500 mL flasks with 400 mL of WAS. The α-amylase was simultaneously added into each flask with the dosage of 0.06 g/g DS. The flasks under strict anaerobic condition were placed in water-bath shakers (100 rpm) for 8 h and the temperature was kept constantly at 40, 50, 60 and 70 °C, respectively. The hydrolysis tests without α-amylase addition were defined as the control tests where other operation parameters were similar to the enzymatic hydrolysis tests. The samples in all flasks were assayed every certain interval.

2.3. Analytical methods

Sludge samples from the reactors were immediately filtered through Whatmann GF/C glass microfiber filter. The filtrate was analyzed for SCOD, NH_4^+–N, carbohydrate and protein, and the filter residue was assayed for TSS and VSS. The TSS, VSS, SCOD and TCOD were determined according to the Standard Methods [15]. Carbohydrate was measured by phenol-sulfuric method with glucose as standard [16]. Soluble protein was determined by Lowry–Folin method with bovine serum albumin (BSA) as standard [17]. Each sample was analyzed in triplicate and the standard deviations of all analyses were always less than 5%, unless noted in the text.

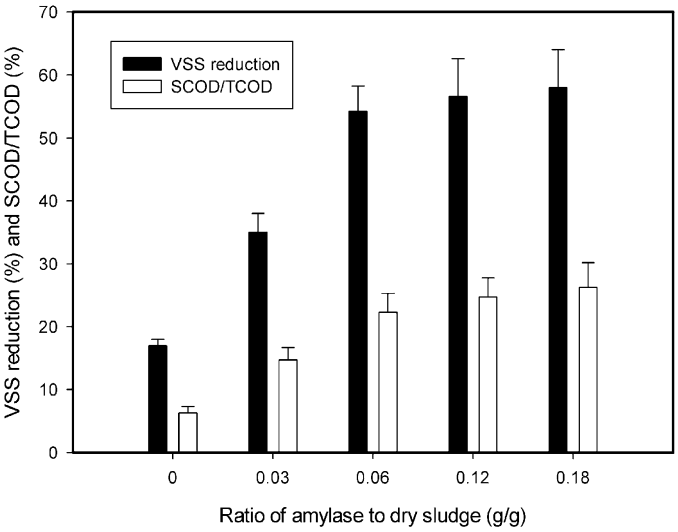

Fig. 1. Effect of α-amylase dosage on WAS hydrolysis (T = 50 °C, t = 4 h).

3. Results and discussion

3.1. Enzymatic hydrolysis process analyzed by ANOVA

As reported previously [8], enzyme dosage and temperature played an important role in WAS hydrolysis. The effects of α-amylase dosage and temperature on WAS hydrolysis was illustrated in Figs. 1 and 2, respectively. The correlations among the two parameters on enzymatic hydrolysis of WAS were assessed and analyzed based on ANOVA using SPSS 13.0 for Windows® (SPSS Inc., 2004). The ANOVA results ($F > F_{0.05}(2, 3)$, $P < 0.05$) indicated significant differences among VSS reduction and SCOD/TCOD ratio at various dosages of α-amylase. Through S–N–K method (Table 1), the five groups (the ratio of α-amylase to DS (g/g) was respectively 0, 0.03, 0.06, 0.12 and 0.18) could be rearranged into three homogeneous groups at significant level of 0.05, which further

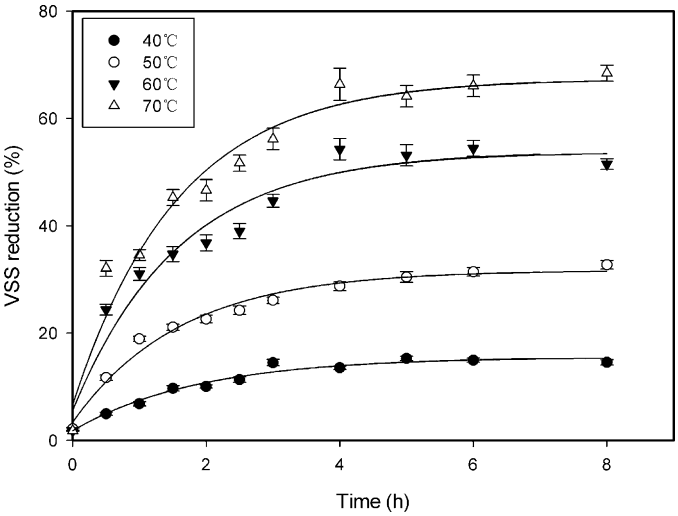

Fig. 2. Variation of VSS reduction at different temperatures (α-amylase = 0.06 g/g DS).

Table 1
Student–Newman–Keuls (S–N–K) analysis for WAS hydrolysis.

VSS reduction	Subset for $\alpha = 0.05$				SCOD/TCOD	Subset for $\alpha = 0.05$			
	N	1	2	3		*N*	1	2	3
1	3	17.0%			1	3	6.3%		
2	3		35.0%		2	3		14.7%	
3	3			54.2%	3	3			22.3%
4	3			56.6%	4	3			24.7%
5	3			58.0%	5	3			26.2%
Sig		1.000	1.000	0.570	Sig		1.000	1.000	0.249

Groups 1–5 represented the ratio of α-amylase to DS (g/g) was 0, 0.03, 0.06, 0.12 and 0.18, respectively.

demonstrated significant differences among the three groups and no significant differences among the third group (the groups 3–5). Based on above analysis, it could be concluded that α-amylase strongly enhanced the WAS hydrolysis, and 0.06 g α-amylase/g DS was obtained as the optimum dosage.

The hydrolysis efficiency of the WAS increased with the temperature increasing from 40 to 70 °C. As depicted in Fig. 2, the VSS reduction was 14.5, 32.7, 51.5 and 68.4%, respectively for 40, 50, 60 and 70 °C at α-amylase dosage of 0.06 g/g DS. The ANOVA results ($F > F_{0.05}(2, 3)$, $P < 0.05$) further indicated a strong impact of temperature on WAS hydrolysis.

3.2. Thermodynamic analysis of WAS hydrolysis

The cumulative effects of different processes taking place during sludge hydrolysis had traditionally been simplified to single first-order kinetics for the substrate biodegradation [2]. The slopes of the curve at different temperatures in Fig. 2 were nearly invariable within the initial 4 h of hydrolysis at α-amylase dosage of 0.06 g/g DS, indicating rapid increase of VSS reduction. Subsequently, the slopes gradually approached stationary value, and only a slight VSS reduction could be observed. According to above description, the hydrolysis of WAS within the initial 4 h could be assumed to obey the following first-order kinetics equations:

$$-\frac{dS}{dt} = kS \quad (1)$$

$$\ln\ S = -kt + b \quad (2)$$

where S was the VSS concentration, k was the rate constant and b was the constant of integration. By plotting $\ln S$ versus t, slope and intercept could be obtained, which corresponded to the value of k and b, respectively. The $\ln S$–t regression curves at different temperatures were illustrated in Table 2.

As shown in Table 2, the k value for α-amylase hydrolysis process increased from 0.106 to 0.215 h^{-1} with the temperature increasing from 40 to 70 °C. Correspondingly, it was respectively 0.007, 0.024, 0.040 and 0.073 (not shown in Table 2) for the control test. Consequently, higher temperature contributed to higher hydrolysis efficiency of WAS, and the α-amylase could accelerate the hydrolysis of WAS. In addition, the goodness of fit values for different temperatures was generally good in the range of 0.92–0.99, which further indicated that the model fitted the experimental data adequately.

Table 2
Kinetic data relevant to VSS hydrolysis enhanced by α-amylase at different temperatures (α-amylase = 0.06 g/g dry sludge).

Temperature (°C)	Dynamic equation	Rate constants k (h^{-1})	Coefficient R^2
40	$y = -0.106x - 0.062$	0.106	0.922
50	$y = -0.142x - 0.076$	0.142	0.925
60	$y = -0.193x - 0.040$	0.193	0.982
70	$y = -0.215x - 0.050$	0.215	0.981

Table 3
Kinetic coefficients in the literature for describing sludge hydrolysis.

Substrate	k (day^{-1})	T (°C)	References
Primary sludge	0.25	35	Siegrist et al. [18]
Primary sludge	0.40	55	Siegrist et al. [18]
Primary sludge	0.169	35	Ferreiro and Soto [19]
Waste activated sludge	0.12	35	Zhang et al. [20]
Waste activated sludge	0.18	55	Zhang et al. [20]
Waste activated sludge	0.026–0.035	35	Tomei et al. [21]
Waste activated sludge	0.17	70	Bolzonella et al. [22]
Waste activated sludge	0.10–0.40	50–65	Ge et al. [23]
Waste activated sludge	0.12–0.80	50–70	Ge et al. [24]
Waste activated sludge	0.017–0.16	10–35	Feng et al. [25]

Table 3 summarized the typical values of rate constant for primary and waste activated sludge that could be found in the literatures. A wide range of values of the first-order rate constants could be seen for the degradation of sludge, which could be explained by different experimental conditions, different ratios of hydrolytic biomass to substrate and the lumped effect of disintegration and hydrolysis.

As we all know, temperature played an essential role in enzymatic hydrolysis of WAS, and its influence was mainly expressed by the rate constant k. According to the Arrhenius equation, the relationship between rate constant k and temperature could be expressed as:

$$\ln\ k = -\frac{E_a}{RT} + \ln A \quad (3)$$

where A was the pre-exponential factor, E_a (kJ/mol) was the reaction activation energy and T (k) was the absolute temperature. The relationship between $\ln k$ and $1/T$ for WAS hydrolysis within the temperature ranges of 40–70 °C was shown in Fig. 3. Obviously, the rate constants for the first-order hydrolysis obtained in this study followed the Arrhenius type of behavior, α-amylase hydrolysis ($\ln k = -2429/T + 5.57$, $R^2 = 0.964$), the control ($\ln k = -7544.0/T + 19.37$, $R^2 = 0.955$).

Veeken and Hamelers [26] studied the temperature dependence of the first-order hydrolysis rate of six solid organic waste components and estimated the average activation energy (64 ± 14 kJ/mol) using Arrhenius equation. In this study, the reaction activation energy for VSS hydrolysis process enhanced by α-amylase and the control test was identified to be 20.19 and 62.72 kJ/mol, respectively. The reaction activation energy for the control test was similar to previous researches [25,26], and approximately three times of the α-amylase treatment, which suggested that α-amylase could increase the rate of chemical reaction largely by lowering reaction activation energy.

3.3. Kinetics analysis of substances conversion during WAS hydrolysis

As reported by Eastman and Ferguson [2], the hydrolysis could be expressed as a first-order process with respect to degradable

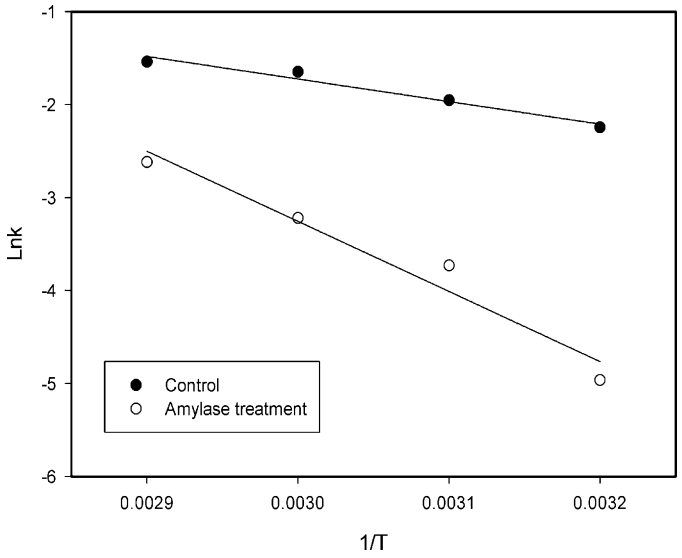

Fig. 3. Relationship between ln k and $1/T$ during WAS hydrolysis.

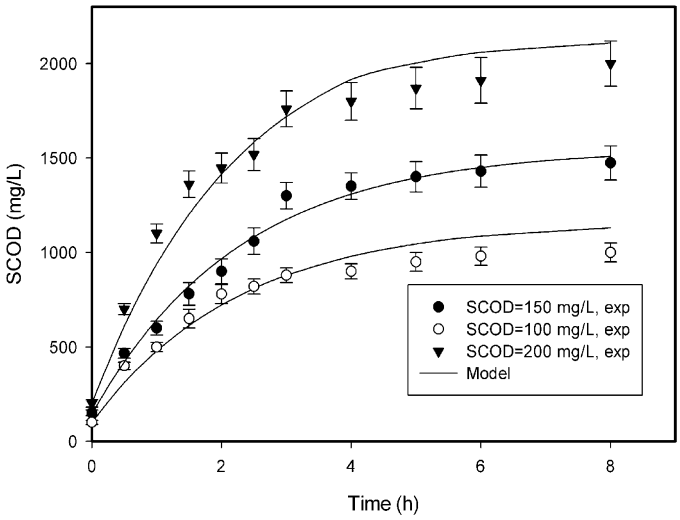

Fig. 4. Comparison between the experimental values and model of SCOD concentration during the WAS hydrolysis at 50 °C.

particulate components. For complex substrate, the first-order kinetics should be modified in order to take the hardly degradable material into account [27]. Although the composition of sludge was complicated, the VSS containing high percentage of organic matter almost took up 70% of the WAS, and it was simply biodegradable material. Therefore, the VSS was chosen as an essential parameter when first-order kinetics was applied in this study. The equations describing hydrolysis as the first-order reaction not directly coupled to the bacterial growth, was expressed as:

$$-\frac{dS}{dt} = kS$$

$$\frac{dX}{dt} = \alpha kS \quad (4)$$

where S, X, k and α represented the VSS concentration, the product concentration, the first-order rate constant and the conversion coefficient of VSS to product. After integrating the above two equations, the product concentration was expressed as:

$$X = X_0 + \alpha S_0(1 - e^{-kt}) \quad (5)$$

where X_0 and S_0 were the initial product and initial substrate concentration, respectively. A non-linear regression could be used to estimate the values of coefficient k, α and their standard deviations.

The primary products of WAS hydrolysis process was soluble monomers which could be measured as soluble COD. Fig. 4 depicted the first-order kinetics of sludge hydrolysis enhanced by α-amylase at different initial waste concentrations at the temperature of 50 °C. The SCOD data corresponding to the initial sludge concentration of 150 mg/L was used to calibrate the first-order kinetics equation and the other two $SCOD_0$ (100 and 200 mg/L) were used to validate the rationality. Substances conversion during WAS hydrolysis enhanced by α-amylase at the temperature of 50 °C was therefore determined. Results from the first-order kinetics fitted the experimental data reasonably well (R^2 = 0.985), and the corresponding substance conversion equation was considered:

$$X = X_0 + 0.266S_0(1 - e^{-0.442t}) \quad (6)$$

The conversion coefficient α predicated from the model (6) was 0.266 ± 0.012 g SCOD/g VSS. As reported by Cirne et al. [28], the substrate conversion coefficient was 0.13 g COD/g VSS for the control reactor, and was 0.23–0.27 g COD/g VSS for enzymatic treatment of municipal solid waste, which was in accordance with our results, and it further indicated that the conversion efficiency of VSS to SCOD was improved through enzymatic hydrolysis.

The enzymatic and thermophilic treatments could improve the dissolution of particulate organic matter to form soluble organics, and carbohydrates could be released by α-amylase. In addition, the α-amylase was an endoglycosidase that hydrolyzed α-1,4-linkages of amylopectin, amylose and glycogen at random positions. The soluble carbohydrate concentration increased from 22.5 to 254.0 mg/L for sludge degradation at 50 °C (Fig. 5), owing to the particulate carbohydrate hydrolysis and release. The lines for soluble carbohydrates released at 50 °C depicted in Fig. 5 fitted to the model (5), and it could be predicated that α = 0.043 ± 0.001 g carbohydrates/g VSS. Fig. 5 displayed that soluble carbohydrates and proteins increased with time up to approximately 4 h, beyond that the two concentrations of products remained constant. Some proteins were released together with carbohydrates when α-amylase was applied during anaerobic digestion, and it was probably due to the nature of EPS and the associated interactions. Carbohydrates might be linked to proteins while others were linked to each other, carbohydrate–carbohydrate, carbohydrate–protein, and protein–protein bonds were possibly formed in this way. Cleavage of one type of bond led to the release of the components that were non-specifically attached to the network [29].

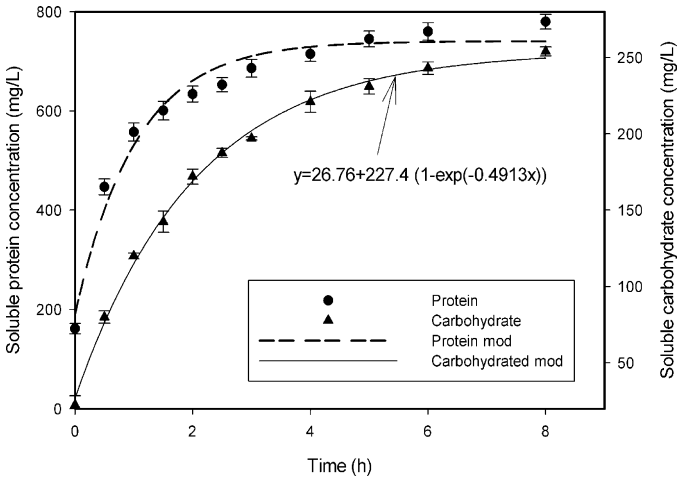

Fig. 5. Comparison between the experimental values and model of protein and carbohydrate concentrations during WAS hydrolysis at 50 °C.

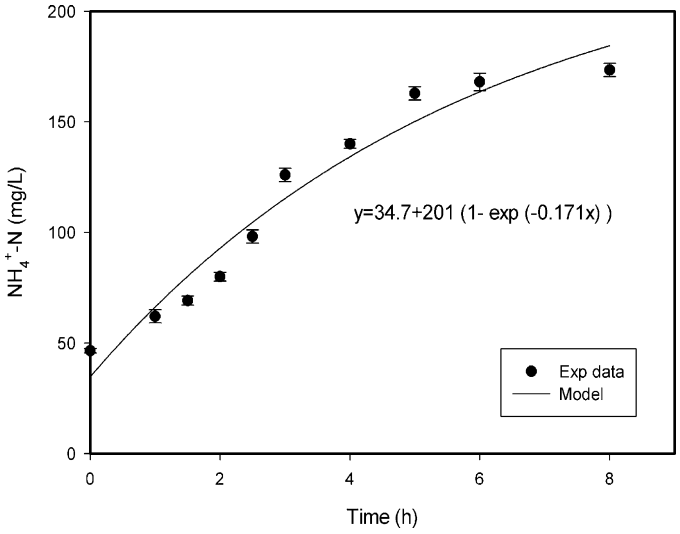

Fig. 6. Time profiles of NH_4^+–N concentrations during WAS hydrolysis at 50 °C.

Nitrogenous organic compounds in the sludge (e.g. protein) were hydrolyzed to NH_4^+–N during pretreatment process, so NH_4^+–N was another important indicator of hydrolysis. The changes of NH_4^+–N during WAS hydrolysis (Fig. 6) well fitted the model (5) and the conversion coefficient α was 0.038 ± 0.002 g NH_4^+–N/g VSS. However, according to Flotats et al. [30], the value was 0.14 g NH_4^+–N/g VS. The distinguished difference should be attributed to different experimental conditions and hydrolysis substrates.

4. Conclusions

In this study, the development of thermodynamics model and the analysis of dynamic behaviors of substances conversion during WAS hydrolysis process enhanced by α-amylase were investigated. Based on ANOVA, the optimum dosage of enzyme was found to be 0.06 g α-amylase/g DS and the temperature was proved to have strong impact on WAS hydrolysis. The influence of temperature on rate constants could be described by Arrhenius equation, and the reaction activation energy for VSS hydrolysis process reduced from 62.72 kJ/mol (control test) to 20.19 kJ/mol (α-amylase treatment). The conversion of various substances during sludge hydrolysis process at the temperature of 50 °C followed first-order kinetics, and the conversion coefficient (α) of VSS to SCOD, carbohydrates and NH_4^+–N was identified as 0.266, 0.043 and 0.038, respectively.

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